

HbA1c measurements from dried blood spots: validation and patient satisfaction

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Abstract

Background: This study evaluates HbA1c measurements from dried blood spots collected on filter paper and compares HbA1c from filter paper (capillary blood) with HbA1c measured in venous blood.

Methods: Patient satisfaction was evaluated using a questionnaire. The performance with the filter paper method was assessed by comparing HbA1c results from EDTA-blood samples obtained via dried blood spots with HbA1c results obtained with freshly hemolyzed blood (routine HbA1c). Adult patients visiting the outpatient clinic for HbA1c analyses were recruited for the evaluation of dried blood spot sampling at home. Laboratory personnel collected a capillary blood sample on filter paper as well as a venous EDTA-blood sample. The participants collected another capillary blood sample at home and sent the dried filter paper back to the laboratory. Samples were analyzed with an immunoturbidimetric assay.

Results: Between-filter coefficient of variation was 1.8%. Filter paper HbA1c increased slightly during storage, particularly during the first 5 days. Filter paper HbA1c highly correlated with routine HbA1c ($r=0.987$). The evaluation of samples collected at home showed comparable HbA1c values by filter paper and routine sampling methods ($n=93$). Eighty-three percent of participants said they would like the filter method to be brought into practice.

Conclusions: Home HbA1c sampling on filter paper is an acceptable sampling alternative for analysis of HbA1c.

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Keywords: capillary blood; dried blood spots; filter paper; HbA1c; patient satisfaction.

Introduction

Diabetes mellitus is a major health problem worldwide. In 2007, it was estimated that ~246 million adults (7.3%) have diabetes mellitus, with 85%–95% having type 2 diabetes. European and North American regions have the highest prevalence at 8.4% and 9.2%, respectively (1). Tight glycemic control of patients with diabetes mellitus is important since it reduces the risk of diabetic complications, including microvascular, neuropathic (2, 3) and macrovascular (4) complications. Glycemic control is monitored by measurement of HbA1c content. HbA1c is the main glycosylated hemoglobin fraction, formed by the irreversible binding of glucose to the N-terminal valine of the β -chains of normal adult hemoglobin (HbA). HbA1c reflects mean blood glucose concentrations over the previous 1–2 months, and forms an integral part of diabetes monitoring (5, 6). It is recommended that HbA1c be measured every 6 months in patients with stable glycemic control, and every 3 months during changes in treatment or for those who do not meet treatment goals (5). The American Diabetes Association recommends maintaining HbA1c as close to normal ($<6\%$) without significant hypoglycemia as optimal (5). However, many official institutes recommend an HbA1c level below 7% as being desirable for adults (6). HbA1c is generally measured when the patient visits his or her physician for evaluation of treatment. Most laboratory HbA1c assays are too laborious to provide fast HbA1c results, and results are usually reported after the patient has visited his doctor. Home sampling devices allow HbA1c samples to be collected, transported and analyzed a few days before a patient visits the physicians' office. These methods allow patients to take their own capillary blood at home, and this eliminates a visit to the laboratory before visiting the physician. Potential devices for collection are EDTA cups or filter paper. Filter paper is easy to handle, can be sent in flat envelopes and is inexpensive. An alternative to home sampling devices is point-of-care testing (POCT). Unlike POCT, filter HbA1c is measured by the same analytical method as measurement performed with venous EDTA-blood for HbA1c. This helps avoid bias between analytical methods and eliminates the need for periodic method comparison studies. This study compares HbA1c from dried capillary blood on filter paper, taken by laboratory personnel and by patients themselves, with HbA1c from freshly hemolyzed venous EDTA-blood samples. We also evaluated the satisfaction of patients with this home HbA1c sampling procedure.

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Patients and methods

Laboratory evaluation protocol

Filters, samples and analytical methods Optimal elution conditions, precision and stability for up to 5 days for HbA1c obtained from dried blood collected on filter papers and the comparability with routine HbA1c analysis was investigated. Filter paper was obtained from Roche Diagnostics (HbA1c via Post[®], Roche Diagnostics GmbH, Mannheim, Germany). Additional stability studies of up to 10 days were investigated with a second type of filter: Whatman 903[®] specimen collection paper (Whatman Nederland BV, Den Bosch, The Netherlands). The Whatman 903 collection paper is imprinted with five half-inch circles and has a wraparound cover to protect the paper before and after sample collection. EDTA-blood samples from anonymized patients were selected based on previously measured HbA1c concentrations. EDTA-blood was applied to the filter paper and allowed to dry at room temperature for at least 3 h. Stored samples were placed in a dark environment. Discs, 3 mm in diameter, were punched out of the filter paper, put in hemolyzing buffer (cat nr. 1488457, Roche, 1 disc per 250 μ L) and were analyzed with the immunoturbidimetric Tinaquant[®] HbA1c assay on a MODULAR P-analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Routine HbA1c measurements were performed by analyzing a mixture of 10 μ L EDTA-blood with 1000 μ L hemolyzing buffer with the Tinaquant[®] HbA1c assay.

The initial laboratory evaluations (precision and stability) employed a 24 h elution period at 4°C, according to elution conditions reported by Jeppsson (7). To improve the logistical processes in our laboratory, we also investigated the possibility of eluting filters for 1, 2, 3 h at room temperature. Filters for studies performed following this evaluation were eluted for 1 h at room temperature (additional stability studies, method comparison and home sampling evaluation).

Elution conditions Ten EDTA-samples were selected and spotted on Post[®] filters. Discs were punched out, placed in hemolyzing buffer and eluted for 24 h at 4°C, or for 1, 2 or 3 h at room temperature prior to HbA1c analyses. A minimal elution period of 1 h was previously reported by Jeppsson (7) using the same filter and elution buffer.

Precision For precision studies, 20 samples were analyzed in duplicate by the routine method, or in duplicate from two Roche filter papers to calculate within- and between-filter precision. Calculations were performed according to CLSI EP5 (8). All discs were eluted for 24 h at 4°C, according to elution conditions reported by Jeppsson (7).

Stability Based on the stability studies of Jeppsson (7), we initially employed a limited study protocol to ensure the stability of HbA1c from dried blood spots. We analyzed blood spots from 10 patients (Roche filters) after storage for 0, 1 and 5 days in the dark at room temperature. Discs were subsequently punched out and left to elute for 24 h at 4°C prior to analyses.

Additional stability studies Stability for up to 10 days was investigated using Whatman 903[®] filter paper. We analyzed blood spots from 10 patients (two filter papers per patient) stored for 0, 1, 5, 7, 8, 9 and 10 days at room temperature. To investigate the influence of exposure of the filter papers to air, one filter paper from each patient was stored exposed to air and the other was stored in a sealed plastic bag. Discs

were punched out and eluted for 1 h at room temperature prior to analysis.

Method comparison We compared HbA1c concentrations using the routine method and using dried blood spots from filter paper. We analyzed the HbA1c concentrations of all EDTA samples collected during one day in our hospital laboratory using the routine whole blood hemolysate and blood collected on filter paper (Roche). Discs were eluted for 1 h at room temperature prior to analysis.

Evaluation of home sampling and patient satisfaction

Participants were recruited from patients visiting the outpatient clinic for HbA1c analysis. Patients aged ≥ 18 years and familiar with taking their own capillary blood samples for home glucose monitoring were eligible for participation. The study was approved by the Medical Ethics Committee of the Medical Center Leeuwarden (The Netherlands). Volunteers gave written informed consent prior to participation. Immediately following collection of a venous EDTA-blood sample for their routine HbA1c analysis, laboratory personnel collected a capillary blood sample which was applied to filter paper and allowed to dry for at least 3 h. Participants were also asked to spot filter paper with capillary blood at home. For this, they received information on the proper procedure for blood collection and a package that contained a filter, envelope and written instructions. Participants were instructed to take a blood sample immediately after arrival at home, to let the filter dry for at least 3 h and to send it back to the laboratory in a sealed plastic cover that same day. On warm days ($> 25^\circ\text{C}$), participants were instructed to post the sample a short time before the mailbox was emptied. Patients also received a questionnaire regarding their satisfaction with the procedure. EDTA-blood samples were analyzed on the day of sampling according to our routine procedure (see procedure above). Following arrival to the laboratory, filters were stored in sealed plastic covers in the dark at room temperature until analysis. All filters were analyzed within 10 days by placing one disc into 250 μ L hemolyzing buffer, and letting the sample elute for 1 h at room temperature.

Data analysis

Data were analyzed using the Analyse-It (Analyse-it software, Ltd., Leeds, UK) and Excel software (Microsoft Corporation, Redmond, WA, US). Precision was calculated using ANOVA [CLSI EP5 protocol (8)] and agreement with Passing and Bablok regression analysis [CLSI EP9 protocol (9)]. Student's t-test was used to investigate differences between groups and paired Student's t-test was used for differences between paired samples. Pearson's correlation coefficient was used to investigate the relationship between HbA1c and any deviation from baseline. $p < 0.05$ was used for statistical significance.

Results

Laboratory evaluation protocol

Elution conditions The mean HbA1c concentration of the selected EDTA samples was 7.2% (range 5.0%–9.3%, $n = 10$). Table 1 shows the relative deviation and the relationship between HbA1c measured from filter paper and our routine HbA1c method

Table 1 Percentage deviation of HbA1c collected on filter paper from venous HbA1c concentrations and the relationship between filter paper and baseline venous HbA1c concentrations.

Elution condition	Bias, % Median [range]	Correlation Pearson R
1 h at RT	-1.4 [-3.3, 2.0]	0.999
2 h at RT	-2.9 [-4.3, 0.0]	0.999
3 h at RT	-3.2 [-6.6, 0.0]	0.997
24 h at 4°C	-6.1 [-11.0, -2.0]	0.995

Ten EDTA-blood samples were spotted and dried on filter paper and then eluted for 1, 2 and 3 h at room temperature, and for 24 h at 4°C. The median [range] relative bias and correlation between filter paper and venous HbA1c is presented in the Table (filter – venous HbA1c). Relationships were evaluated using Passing and Bablok regression analyses and the Pearson correlation coefficient.

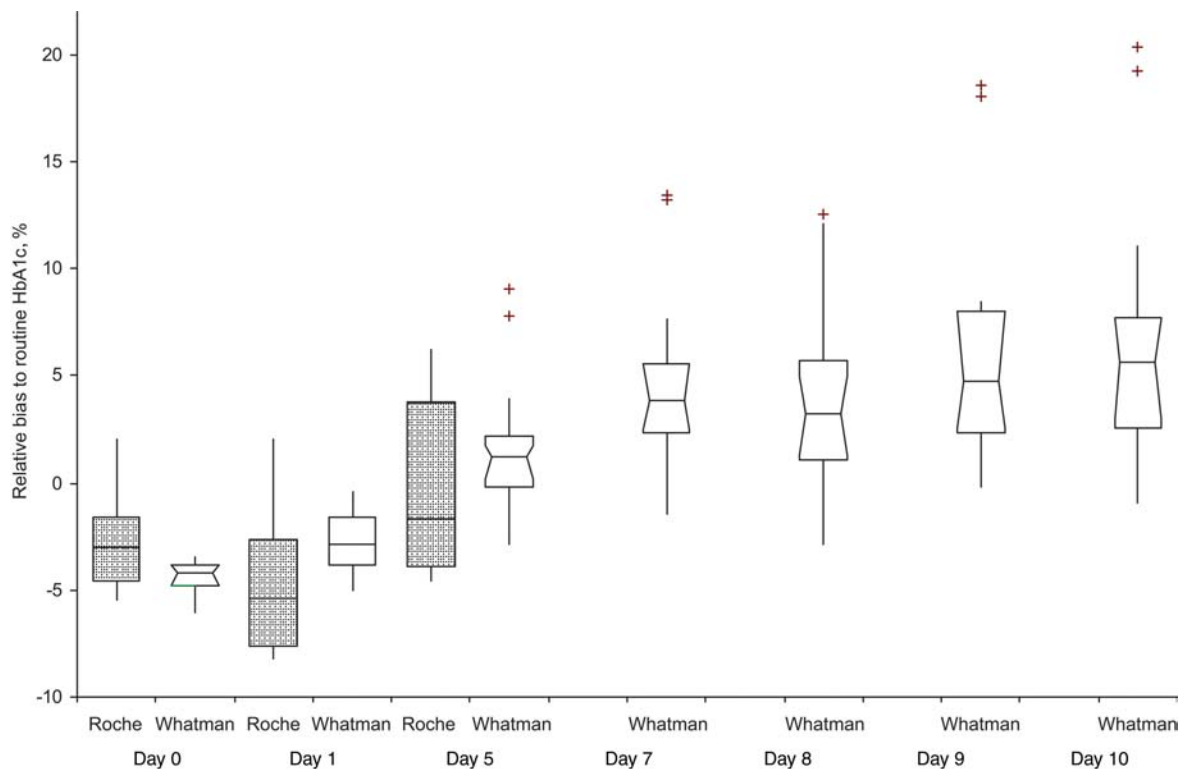
according to the different elution conditions. HbA1c concentrations from filter paper agreed most closely with HbA1c concentrations measured using our routine method when filter paper was eluted for 1 h at room temperature (RT).

Precision The 20 EDTA samples that we selected had a mean HbA1c concentration of 7.2% (range 4.8%–10.9%) when measured using our routine HbA1c method. The precision of duplicate HbA1c measurements using our routine analysis method was 1.1%. Within-filter paper variation was 1.1% and between-filter variation was 1.8%. Measurement of HbA1c quality control samples using our routine HbA1c analysis over a 2-month period showed between-run coefficients of variation of 1.6% (n=46,

mean HbA1c concentration=5.41%), 1.6% (n=46, mean=7.51%) and 1.3% (n=46, mean=10.65%).

Stability The first stability study showed that HbA1c concentrations increased in the dried blood spots from days 0 to 5 (Figure 1). Filter paper HbA1c was lower compared with routine HbA1c measurements when analyzed on days 0 and 1 ($p<0.05$), but not on day 5.

Additional stability studies Stability results with the Roche filter paper were confirmed with additional stability studies using the Whatman 903® filter paper. HbA1c concentrations increased in dried blood spots from days 0 to 10, with the largest increases from day 0 to 5 (Figure 1). These changes resulted in a median

**Figure 1** Stability studies.

The Figure displays boxplots of the relative bias to routine HbA1c analysis of HbA1c from dried blood spots stored up to 10 days in the dark at room temperature. Roche=primary stability study, 10 patients, filter papers stored for 0, 1 and 5 days. Whatman=additional stability study, 10 patients (2 filter papers per patient), filter papers stored for 0, 1, 5, 7, 8, 9, 10 days. The boxes represent medians and interquartile ranges. Outliers are shown as “+” and defined as observations which lie more than $1.5 \times$ the interquartile range (IQR) lower than the first quartile or $1.5 \times$ IQR higher than the third quartile.

between-day variation of 3.5% [range 1.0%–8.0% (maximum 6.2%, excluding outliers)]. Median between-day variation for filters stored 5–10 days was 1.7% [0.7%–5.0% (0.7%–3.0%, excluding outliers)]. Filter paper HbA1c was lower than routine HbA1c when analyzed on days 0 and 1, and was higher on days 5–10 ($p < 0.05$ for all). Increases in HbA1c were similar for filters stored exposed to air compared with filters stored in a sealed plastic bag (all days $p > 0.05$). A positive correlation was shown between routine HbA1c measurements and the deviation in filter paper HbA1c concentrations from routine HbA1c concentrations during days 5–10, but not at days 0 and 1. (Spearman correlation, $p < 0.05$ for all).

Method comparison The 73 samples had a mean HbA1c concentration of 6.7% (range 4.7%–9.3%) by routine HbA1c analysis. HbA1c concentrations measured using filter paper showed significant correlation with our routine HbA1c method (Pearson $r = 0.987$). The intercept $[-0.011, 95\% \text{ confidence interval (CI): } -0.232, +0.183]$ was not significantly different from zero, and the slope (0.987, 95% CI: 0.957, 1.019) was not statistically different from 1.

Evaluation of home sampling and patient satisfaction

The entire study group was comprised of 93 participants, 54 males and 39 females. The mean age was 59.1 years (range 23–85 years). Five participants, two males and three females, did not return the home sampling filter paper, and four did not return the questionnaire. Since participants gave written informed consent, their HbA1c concentrations measured using the laboratory filter paper and our routine method were included in the statistical analysis. Twenty-six percent of participants were reported to have diabetes mellitus type 1, 64% type 2 and 10% did not know their diabetes type. Participants had diabetes for 0.5–43 years (median 8.0 years). Filter papers collected at home were analyzed within 10 days after sampling. All samples were sent when outdoor temperatures ranged from -9 to 11°C , and were stored at 19 – 23°C .

Figure 2 shows the comparison between the routine HbA1c method and the filter paper method, where “filter lab” indicates blood that was collected by laboratory personnel and “filter home” indicated blood that was collected at home. Passing and Bablok regression analysis revealed that both intercepts did not significantly deviate from zero and both slope values were not statistically different from 1. No significant bias was observed; $+0.011$ (95% CI: $-0.037, +0.060$) between samples collected at home and -0.025 ($-0.072, +0.021$) samples collected in the lab with respect to measured HbA1c concentrations. The Pearson correlation coefficients were 0.980 ($p < 0.0001$) for both comparisons. The Pearson correlation coefficient for the comparison between “filter lab” and “filter home” was $r = 0.977$ ($p < 0.0001$).

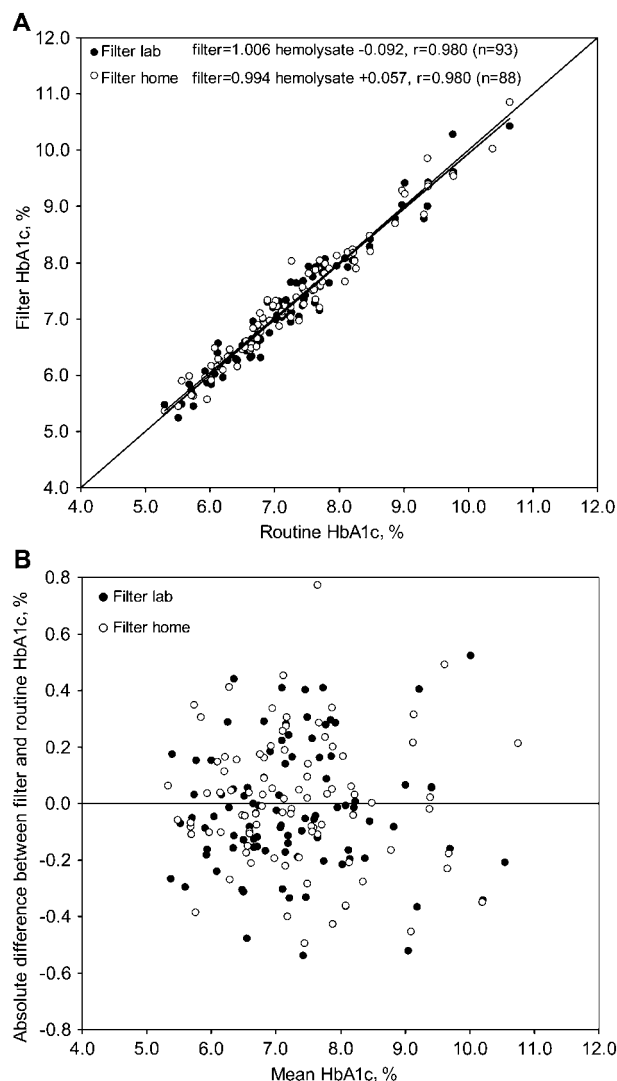


Figure 2 Passing and Bablok regression analysis (A) and bias plots (B) for home and lab filter paper HbA1c vs. HbA1c from hemolysate.

The dashed line in Figure 2A indicates perfect agreement between x and y. The two regression lines for home and lab filter paper HbA1c are black lines. These are difficult to discriminate, since these overlap to a large extent.

The questionnaire revealed that many patients were satisfied with the filter paper method. Of the participants that responded, 90% indicated that the search for an alternative method for sampling is valuable, 94% indicated that the instructions for sampling were understandable, 97% said that collection with filter paper was easy, 96% were satisfied with home collection, 83% indicated that home collection in the future is desirable and 83% said that the filter paper method of collection should be brought in practice.

Discussion and conclusions

We evaluated the performance of dried blood spots for analysis of HbA1c and investigated patient satisfaction with home collection of HbA1c samples. The first report on measurement of glycated protein from

dried blood spots was published in 1982 by Goldstein et al. (10). Since then, others have also demonstrated that HbA1c can be measured using dried blood spots with acceptable accuracy and precision by use of ion-exchange chromatography (7, 11–15), affinity chromatography (9, 13), a modified barbituric acid method (16) and by use of immunoturbidimetric assays (7, 17). The stability of HbA1c in dried blood samples is the major concern. HbA1c may be formed by in vitro glycation of hemoglobin, and may also be degraded during storage in dried blood (18). Our results illustrate that pre-analytical factors need to be taken into account. In particular, storage and elution conditions were important factors. Storage increased HbA1c concentrations, whereas prolonged elution times decreased HbA1c. Both effects may contribute significantly to the pre-analytical variation. It has been shown previously that high blood glucose concentrations increase glycated protein in dried blood samples (16). Our results confirm this finding, especially when one considers the positive relationship between HbA1c concentrations and the changes in HbA1c during storage. The increase may be greater at higher temperatures (14), although no temperature effect was evident in the study by Jeppsson et al. (12). The in vitro effects may also depend upon the analytical method that is used to measure HbA1c (18). Large clinical trials, such as the Diabetes Control and Complications Trial (2) and the United Kingdom Prospective Diabetes Study (3), have shown convincingly that an absolute HbA1c difference of 1% is related to a significant reduction in risk from microvascular as well as macrovascular diabetic complications. Within a single patient, an absolute change of 1% is also considered to be significant. The within-person biological variation of HbA1c in non-diabetic individuals is estimated to be very small. These biological factors demand strict analytical criteria. Currently, the recommended desirable goal for analytical precision is <3%, with an optimum goal of <2% (19). Standardization of procedures and evaluation of the filter paper method in daily practise is therefore important. Another option is the use of filter paper pretreated (13–16, 20) with, for example, glucose oxidase. These have been shown to enhance the stability of HbA1c when compared to untreated filter paper. The relative instability and subsequent variability did not seem to be a major problem when using filter paper in our daily practise, given the good agreement between home filter paper HbA1c and routine HbA1c analyses (Figure 2). In addition, filter paper HbA1c analyzed between days 5 and 10 after collection had a median total analytical variation of 1.7%, which is comparable to the analytical variation of routine HbA1c analysis. This figure compares favorably to the 2.7% for 3–10 days storage of the pretreated filter papers that were stored with an unknown patented solution in the study of Parkes et al., who used a similar method of analysis (20).

A review of six studies (21) demonstrated that immediate availability of HbA1c improves diabetes management, results in lower HbA1c concentrations

on subsequent visits and influenced clinical management in most studies. Kennedy et al. (22) performed a large randomized controlled study with 7893 adults and showed that point-of-care measurement of HbA1c resulted in a significantly higher percentage of patients achieving HbA1c concentrations below 7%. Our study showed that many patients supported the search for an alternative method of collection, and were satisfied with the filter paper method tested. Patients are able to send their blood sample by post, and this method provides an opportunity for the physician to choose whether he or she wishes to examine stable patients during a hospital visit. We conclude that home collection of blood for HbA1c on filter paper is an acceptable, easy and inexpensive alternative for collection of blood samples for HbA1c analysis when pre-analytical factors are taken into account.

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